

## Astrovirus, Reovirus, and Rotavirus Concomitant Infection Causes Decreased Weight Gain in Broad-Breasted White Poults

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**SUMMARY.** Turkey astrovirus type-2 (TAsV-2), turkey rotavirus (TRotV), and turkey reovirus (TReoV) have been implicated as possible causes of enteric diseases and poor production in turkeys; however, numerous studies with each individual virus have failed to reproduce the disease as observed in the field. Therefore, in this study we evaluated the pathogenesis of all possible combinations of one, two, or three viruses in comparison to sham inoculates in 3-day-old turkey poults. Body weights were recorded at 2, 4, 7, 10, and 14 days postinoculation (PI) and were decreased in virus-infected turkeys throughout the experiment as compared to sham inoculates. Although not significantly different from the other virus-exposed groups, the poults exposed to all three viruses had the lowest body weights throughout the experiment. Clinical signs, including huddling, diarrhea, and agitation, were only observed in groups exposed to TAsV-2 and/or TRotV. At 4 days PI, birds from each treatment group were necropsied, and pale intestines with watery contents and undigested feed were observed in the groups that were exposed to TRotV + TReoV or TRotV + TAsV-2 and the group exposed to all three viruses. Minimal microscopic lesions were observed in the intestines of turkeys infected with TAsV-2, TReoV, or a combination of both. In the turkeys infected with TRotV, either alone or in combination with other viruses, mild microscopic lesions were found in all sections of the small intestine and viral antigen was identified by immunohistochemical staining in mature enterocytes. No or very mild lesions were observed in other organs with the exception of the bursa of Fabricius, where mild to severe atrophy was observed in all virus-infected poults examined. Cloacal shedding of TAsV-2 and TRotV was evaluated by reverse-transcription PCR testing of cloacal swabs and minimal differences were observed among the treatment groups.

**RESUMEN.** Descenso en la ganancia de peso de pavipollos blancos de pechuga amplia por la infección simultánea de astrovirus, reovirus y rotavirus.

El astrovirus de los pavos tipo 2 (TAsV-2), el rotavirus del pavo (TRotV), y el reovirus de los pavos (TReoV) han estado implicados como posibles causas de enfermedad y baja producción en pavos. Sin embargo, numerosos estudios utilizando cada virus individualmente, han fracasado en la reproducción de la enfermedad tal como se presenta en el campo. Por lo tanto, en este estudio, se evaluó la patogénesis de todas las combinaciones posibles entre uno, dos ó tres virus en pavipollos de tres días de edad y se compararon con aves controles negativos. Se registraron los pesos corporales a los 2, 4, 7, 10, y 14 después de la inoculación y se observó disminución en el peso en las aves inoculadas durante el periodo experimental en comparación con las aves controles negativos. A pesar de que no existieron diferencias significativas con otros grupos con exposición viral, los pavipollos expuestos a los tres virus de manera simultánea, presentaron los pesos corporales más bajos a lo largo del experimento. Los signos clínicos incluyeron, amontonamiento entre las aves, diarrea y agitación, que fueron observados con los grupos expuestos a los virus TAsV-2 y/o TRotV. A los cuatro días postinoculación, se practicó la necropsia en las aves de cada grupo experimental, se observó palidez en intestinos con contenidos acuosos y alimento sin digerir en los grupos expuestos a los virus TRotV junto con TReoV o en las aves expuestas a TRotV junto con TAsV-2 y en el grupo expuesto a los tres virus. Se observaron lesiones microscópicas mínimas en los intestinos de pavos infectados con TAsV-2, TReoV, o con la combinación de los dos. En los pavos infectados con TRotV, solo o en combinación con los otros virus, se encontraron lesiones leves en todas las secciones del intestino delgado y el antígeno viral se identificó mediante inmunohistoquímica en enterocitos maduros. En otros órganos, no se observaron lesiones o éstas fueron mínimas exceptuando la bolsa de Fabricio, donde se observó atrofia leve a severa en todos los pavipollos infectados que fueron examinados. Se evaluó la eliminación cloacal de TAsV-2 y TRotV mediante la transcripción reversa y reacción en cadena de la polimerasa con hisopos cloacales y se observaron diferencias mínimas entre los grupos tratados.

**Key words:** astrovirus, enteric disease, reovirus, rotavirus, turkeys

**Abbreviations:** HRP = horseradish peroxidase; IHC = immunohistochemical staining; PEC = poult enteritis complex; PEMS = poult enteritis mortality syndrome; PI = postinoculation; RT-PCR = reverse transcription-PCR; TAsV = turkey astrovirus; TCID<sub>50</sub> = 50% tissue culture infectious doses; TReoV = turkey reovirus; TRotV = turkey rotavirus

Enteric disease in turkeys has an important economic impact because of production losses due to poor feed conversions and poor weight gain. Sporadic outbreaks of enteric disease are seen worldwide in commercial poultry and can vary widely in severity (reviewed by Reynolds [13]). The causes of enteric disease have never been definitively established because they are complex and polymicrobial and similar disease signs can likely be caused by different pathogens. Viruses from numerous virus families have been involved in causing

enteric disease including avian astroviruses, particularly turkey astrovirus type 2 (TAsV-2) (10,14,26), avian reoviruses (6,7,18), and avian rotaviruses (23,24). However, because experimental infections with individual viruses are rarely reported to reproduce disease with the same severity observed in the field and surveys of chickens and turkeys have shown that infection with these viruses in “healthy” flocks is not uncommon (8), it has been difficult to definitively identify what is necessary and sufficient to cause poult enteritis complex (PEC) and poult enteritis mortality syndrome (PEMS).

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Table 1. Mean body weight in grams  $\pm$  standard deviation by treatment group and days postinoculation. Values followed by different letters have statistically significant ( $P < 0.05$ ) differences in body weights.

Treatment group <sup>A</sup>	Days postinoculation					
	0	2	4	7	10	14
Sham	53.4 $\pm$ 5.2a	73.5 $\pm$ 7.0a	99.1 $\pm$ 11.4a	149.4 $\pm$ 16.1a	252.0 $\pm$ 34.9a	350.0 $\pm$ 54.9a
TRotV	49.9 $\pm$ 3.7a	62.5 $\pm$ 5.9bc	81.5 $\pm$ 11.3c	128.3 $\pm$ 22.0bc	200.1 $\pm$ 34.5b	282.0 $\pm$ 45.9b
TAstV-2	50.2 $\pm$ 4.1a	66.4 $\pm$ 4.2bc	89.8 $\pm$ 8.5bc	126.8 $\pm$ 17.6bc	192.1 $\pm$ 30.9b	267.5 $\pm$ 45.3b
TReoV	50.1 $\pm$ 3.6a	68.0 $\pm$ 5.5b	95.5 $\pm$ 7.8ab	139.9 $\pm$ 17.7a	216.0 $\pm$ 32.2b	298.6 $\pm$ 45.7b
TRotV + TReoV	51.1 $\pm$ 3.8a	64.8 $\pm$ 5.7bc	80.0 $\pm$ 16.8c	136.7 $\pm$ 9.9ab	221.9 $\pm$ 25.8ab	303.5 $\pm$ 41.1ab
TRotV + TAstV-2	49.5 $\pm$ 2.8a	63.6 $\pm$ 3.4bc	81.0 $\pm$ 7.3c	124.7 $\pm$ 13.6bc	204.5 $\pm$ 19.5b	282.0 $\pm$ 29.3b
TAstV-2 + TReoV	49.3 $\pm$ 4.7a	61.6 $\pm$ 8.5bc	82.2 $\pm$ 11.6c	119.0 $\pm$ 19.9bc	195.7 $\pm$ 34.0b	273.1 $\pm$ 47.9b
TRotV + TAstV-2 + TReoV	50.0 $\pm$ 4.3a	60.2 $\pm$ 6.0c	72.1 $\pm$ 9.6c	111.6 $\pm$ 20.2c	189.2 $\pm$ 31.0b	263 $\pm$ 44.0b

<sup>A</sup>TAstV-2 = turkey astrovirus type-2; TRotV = turkey rotavirus; TReoV = turkey reovirus.

Since numerous surveys of virus incidence have shown that turkey flocks are frequently infected with both an astrovirus and a rotavirus (8,15,16), we evaluated the pathogenesis of concomitant infection of young turkey poults with both TAstV-2 and a turkey-origin rotavirus (TRotV) to determine if there is a synergistic interaction between the viruses. In addition, a turkey reovirus (TReoV) isolated from turkeys with enteric disease that has been shown to be immunosuppressive (4) was included as a third virus. Here we report the pathogenesis of isolates of TAstV-2, TRotV, and TReoV in all possible combinations in 3-day-old broad-breasted white poults. To our knowledge pathogenesis studies with all three viruses in all combinations have not been previously reported.

## MATERIALS AND METHODS

**Viruses.** All three viruses were originally isolated from turkey flocks with unevenness and/or diarrhea. The TAstV-2 isolate was Turkey/CA/SEP-270/04 (10) and was propagated for no more than three passages in embryonated turkey eggs. The TRotV isolate was Turkey/MN/SEP-996/07, which was isolated in MA-104 cells using previously reported methods (22) and was passaged three times in MA-104 cells. The TReoV isolate was Turkey/NC/SEP-R44/03 (20), which was passaged in VERO cells four times. TRotV and TReoV were titrated in cell culture using the same cell lines as those used for virus propagation as described previously (20) by inoculating the cells with 10-fold dilutions of virus prepared in serum-free media. Cells were observed for cytopathic effects to evaluate infection status. TAstV-2 was titrated in embryonated turkey eggs as previously described (10). Titers were calculated with the Reed–Muench method (12).

The TAstV, TReoV, and TRotV isolates used here were each tested for the following adventitious agents (excluding homologous viruses): adenovirus, astroviruses (including avian nephritis virus and turkey-astrovirus type-1 and type-2), coronavirus, reovirus, and rotavirus by PCR or reverse transcription (RT)–PCR (3,19,21) and were found to be negative.

**Experimental infection of turkeys.** Broad-breasted white turkeys were obtained from a commercial hobby-bird hatchery at 2 days post-hatch. The absence of TRotV, TReoV, and TAstV-2 infection were confirmed with RT-PCR methods as described previously (3,19). The poults were divided into eight treatment groups of 12 (Table 1) and housed in Horsfall isolators with *ad libitum* access to feed and water. Animals were humanely cared for in accordance with established institutional animal care and use procedures.

At 3 days posthatch the poults were inoculated with 0.2 ml of the appropriate material by oral gavage. The virus doses were TAstV-2 Turkey/CA/SEP-270/04  $10^7$  50% egg infectious doses, TRotV Turkey/MN/SEP-996/07  $10^3$  50% tissue culture infectious doses (TCID<sub>50</sub>), and TReoV Turkey/NC/SEP-44/03  $10^4$  TCID<sub>50</sub>. The highest dose possible was administered for TRotV and TReoV since these viruses do not grow to high titers in cell culture.

All birds were weighed and cloacal swabs were collected at 0, 2, 4, 7, 10, and 14 days postinoculation (PI) to evaluate virus shed by RT-PCR. The cloacal swabs were collected in phosphate buffered saline and frozen at  $-70^\circ\text{C}$  and later tested by RT-PCR for each virus to which the birds were exposed. On day 4 PI, two birds from each group were necropsied and bursa, liver, spleen, thymus, proventriculus, ceca, jejunum, ileum, and duodenum/pancreas were collected in 10% neutral buffered formalin for microscopic evaluation. At 21 days PI, serum was collected from the remaining birds. Since RT-PCR was not used with TReoV, infection was confirmed by homologous virus neutralization assay conducted as previously described (20) using serum collected from surviving birds at the termination of the experiment. Serum collected from five poults prior to inoculation was also tested for TReoV antibody and found to be negative.

**Real-time RT-PCR and conventional RT-PCR.** RT-PCR methods were used to evaluate cloacal shed of TAstV-2 and TRotV (TReoV was not included in the RT-PCR testing because it does not replicate well in the intestine and is shed poorly by the cloacal route) (9,19). Samples were tested for TAstV-2 by a real-time RT-PCR assay that targets the ORF 1B (POL gene) (19). TRotV was tested for with a conventional RT-PCR test that targets the NSP4 gene (3).

**Statistical analysis of body weights.** Body weights were compared among all groups by one-way repeated measures ANOVA (Student–Newman–Keuls method) (SigmaStat 3.1, Systat Software, Richmond, CA). A  $P$  value of  $<0.05$  was considered to be significant.

**Histopathology.** Tissues were paraffin-embedded, sectioned, mounted, stained with hematoxylin and eosin, and examined by light microscopy. All samples were evaluated in a manner that blinded the examiner to the treatment groups.

**Immunohistochemical staining (IHC) for the detection of TRotV in tissues.** We focused on TRotV for the IHC assay because we have previously reported IHC results with the TAstV-2 and TReoV isolates utilized here (9,10). A peroxidase immunohistochemical technique using convalescent or hyperimmune sera from rotavirus-inoculated turkeys or chickens was used to identify rotavirus in all the tissues collected (bursa, liver, spleen, thymus, proventriculus, ceca, jejunum, ileum, and duodenum/pancreas) from infected poults. All procedures were done at room temperature except when noted. Tissue sections were cut (4  $\mu\text{m}$  thick) from paraffin-embedded tissue samples and mounted on charged glass slides (Superfrost/Plus; Fisher Scientific, Suwanee, GA). Deparaffinization, antigen retrieval, and blocking procedures have been previously described (9).

To ensure consistency, antibodies from two sources were utilized separately as primary antibodies: 1) turkey convalescent sera collected at 21 days PI as described above, and 2) sera from 14-wk-old chickens hyperimmunized with beta-propiolactone inactivated TRotV Turkey/MN/SEP-996/07. The primary antibodies were each diluted 1:100 in streptavidin peroxidase antibody diluent (Biogenex, San Ramon, CA) before use. Slides were incubated with the primary antibody overnight at  $4^\circ\text{C}$ . The secondary antibody was goat anti-turkey IgG, horseradish peroxidase (HRP) labeled or goat anti-chicken IgG, HRP labeled (Southern Biotechnology Associates, Inc., Birmingham, AL), diluted

Table 2. Distribution and severity of histological lesions in poultlets inoculated with turkey astrovirus type-2 (TAsV-2), turkey rotavirus (TRotV), or turkey reovirus (TReoV) and all combinations at 4 days postinoculation.

	TRotV	TRotV + TAsV-2	TAsV-2	TRotV + Reo	Reo	TAsV-2 + Reo	TRotV + Reo + TAsV-2
Duodenum	+ <sup>A</sup>	+	—	+	—	+/-	+
Jejunum	+	+	+/-	+	+/-	+/-	+
Ileum	+	+/-	—	+/-	—	—	+/-
Bursa	+/-	+	++	++	++	+++	+++

<sup>A</sup>Microscopic lesions: — = no or sporadic lesions; +/- = minimal; + = mild; ++ = moderate; +++ = severe.

1:100 in antibody diluent (Biogenex), and incubated for 1 hr. The reaction was visualized with the DAB Substrate kit for peroxidase (Zymed Laboratories Inc., San Francisco, CA). After staining, the sections were counterstained with hematoxylin, air dried, cover-slipped, and examined by light microscopy. Tissues collected from sham inoculated poultlets served as negative controls. Additional primary antibody controls included nonimmune goat sera, and turkey convalescent antiserum specific for TReoV used in place of the TRotV-specific antibody (9).

## RESULTS

**Body weights.** At 2 days PI, the mean body weights of all treatment groups were significantly different from the sham inoculated group. The TAsV-2 alone, TRotV alone, and TRotV + TAsV-2 groups and the group exposed to all three viruses remained significantly different from the sham inoculates for the duration of the experiment (Table 1). The TReoV only group body weights were similar to those of the sham inoculates at 4 and 7 days PI and the TRotV + TReoV group body weights were similar to the sham inoculates at 7, 10, and 14 days PI. Among the treatment groups exposed to viruses there was no group that was significantly different from the other virus infected groups at all sample times, although the group exposed to all 3 viruses did have the lowest body weights at all time points postinfection.

**Clinical sign and gross lesions.** Clinical signs which included huddling, diarrhea and agitation/increased vocalization, were observed 4–11 days PI, and were generally mild and similar among the groups exposed to either TAsV-2 or TRotV. Clinical signs were not observed in the sham inoculates or the group exposed to TReoV only. The severity of clinical signs was not clearly greater in any of the groups exposed to 2 viruses or all 3 viruses as compared to the groups exposed to a single virus. Mortality was minimal throughout the experiment; at 4 days PI one TRotV only exposed bird died, at 5 days PI one sham inoculate died and at 6 days PI one TRotV + TReoV-exposed bird died.

At necropsy at 4 days PI, gross lesions consisting of pale or translucent intestines with watery contents and undigested feed were seen in all birds from the groups inoculated with TRotV + TReoV, TRotV + TAsV-2, and TRotV + TReoV + TAsV-2. Gross lesions were not observed in any of the other groups.

All of the remaining TReoV-exposed birds were tested for antibody to confirm infection at the termination of the experiment by virus neutralization assay and all were positive.

**Microscopic lesions.** The distribution and severity of histological lesions by tissue and virus are summarized in Table 2. No lesions were observed in tissues collected from sham inoculated poultlets at any time. The cecum, liver, pancreas, kidney, thymus, and proventriculus, which presented no lesions or very mild lesions in all treatment groups, were excluded from the table. Intestinal sections were scored for degeneration, vacuolation, and sloughing of intestinal epithelial cells; villous atrophy, blunting, and fusion; crypt epithelial hyperplasia; and infiltration of the lamina propria with inflamma-

tory cells. Bursa sections were scored for lymphoid depletion and fibroplasia. Lesions were scored in increasing levels of severity as follows: absent or sporadic, minimal, mild, moderate or severe.

Mostly minimal lesions were present in the intestines of turkeys infected with TAsV-2, TReoV, or a combination of both. Shrunken degenerate cells present in the villi and crypt epithelium and mild crypt hyperplasia resulting in increased crypt depth were the most common histopathological findings. Mild villus shortening and increased number of lymphocytes in the lamina propria were also present. These lesions occurred principally in the jejunum.

In the turkeys infected with TRotV, alone or in combination with other viruses, mild microscopic lesions were found in all sections of the small intestine. Villous atrophy with widening of the lamina propria and separation of enterocytes and desquamation at the distal third of the villi was observed (Fig. 1). Increased crypt depth with marked hypercellularity and numerous mitotic figures was present principally in the jejunum and duodenum. Vacuolated and degenerated enterocytes were commonly found in the crypt.

Bursal atrophy characterized by lymphoid depletion and fibroplasia were the major histological lesions associated with TReoV infection in poultlets. TAsV-2 also induced mild bursal atrophy, and the combination of all three viruses induced the most severe bursal damage with severe fibroplasia between bursal follicles (Fig. 2). Mild to moderate lymphoid depletion and histiocytic and ellipsoidal hyperplasia were present in the spleens of all infected turkeys. Mild lymphocytic infiltration was present in the liver and pancreas of some infected birds.

**Detection of rotavirus viral antigen by IHC.** Intestinal tissue from TRotV-infected poultlets, but not those from the control poultlets, contained detectable viral antigen. Positive staining by IHC was characterized by the presence of intracytoplasmic dark brownish granules. No specific peroxidase-positive staining was seen in the tissues of the control poultlets. Of the two primary antibodies used, the chicken hyperimmune sera gave the best results, with less background and stronger staining. In spite of the difference in quality of staining, both antibodies gave the same staining pattern. Positive staining was observed in the cytoplasm of the mature enterocytes at the tip of the affected villi (Fig. 1). Staining was present in all sections of the small intestine, but was observed more frequently in the jejunum and duodenum. No specific staining was observed in the bursa, liver, spleen, thymus, or proventriculus.

**RT-PCR on cloacal swabs.** Real-time RT-PCR and conventional RT-PCR on cloacal swabs was used to evaluate cloacal shed of TAsV-2 (Table 3) and TRotV (Table 4). The poultlets used in this study were negative for TAsV-2 and TRotV when they arrived from the hatchery. Infection with more than one virus did not clearly affect virus shed for either TAsV-2 or TRotV; however, TAsV-2 was generally shed for a longer period of time than TRotV. Briefly, all birds in treatment groups that received TAsV-2 were positive for TAsV-2 from 2 through 11 days PI, except TAsV-2 + TReoV at 4 days PI, for which only 80% of the poultlets were positive. At 14 days PI, the percentages of TAsV-2-positive poultlets in each group were 40% in TAsV-2 only, 60% in TRotV + TAsV-2, 70% in the group

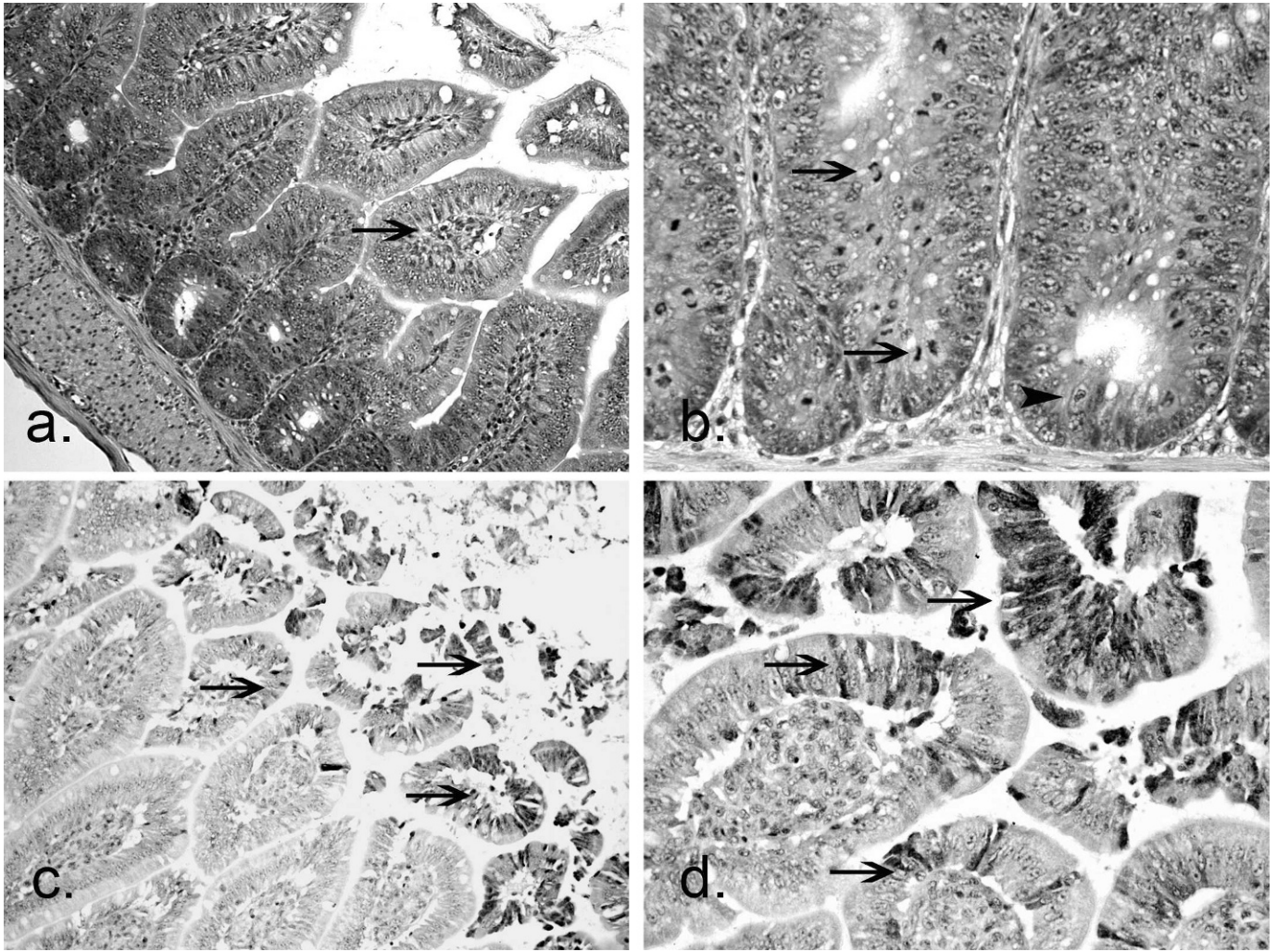


Fig. 1. Photomicrographs of sections of the jejunum from a poult infected with rotavirus, 4 days PI. (a) Widening of the lamina propria of the villi (arrow) and increased crypt depth. Hematoxylin and eosin. Magnification, 200 $\times$ . (b) Marked hypercellularity and numerous mitotic figures (arrows) and vacuolated enterocytes (arrowhead). Hematoxylin and eosin. Magnification 400 $\times$ . (c) and (d) Separation of enterocytes from the lamina propria and desquamation at the distal third of the villi. Viral antigen staining present in the cytoplasm of the enterocytes at the distal section of the affected villi (arrows, dark staining). Immunoperoxidase labeling, hematoxylin counter stain. Magnification, 200 $\times$  and 400 $\times$ , respectively.

exposed to all three viruses, and 87.5% in the TAstV-2 + TreoV group.

Cloacal swabs from all poults that received TRotV were positive TRotV at 2 days PI (Table 4). At 4 days PI, TRotV was only detected

in swabs from 45% of the birds exposed to all three viruses, 50% of the TRotV + TAstV-2 birds, 60% of the TRotV + TReoV birds, and 70% of the poults in the TRotV only group. By 7 and 11 days PI, TRotV was only detected in 10%–25% of the poults, and at 14 days

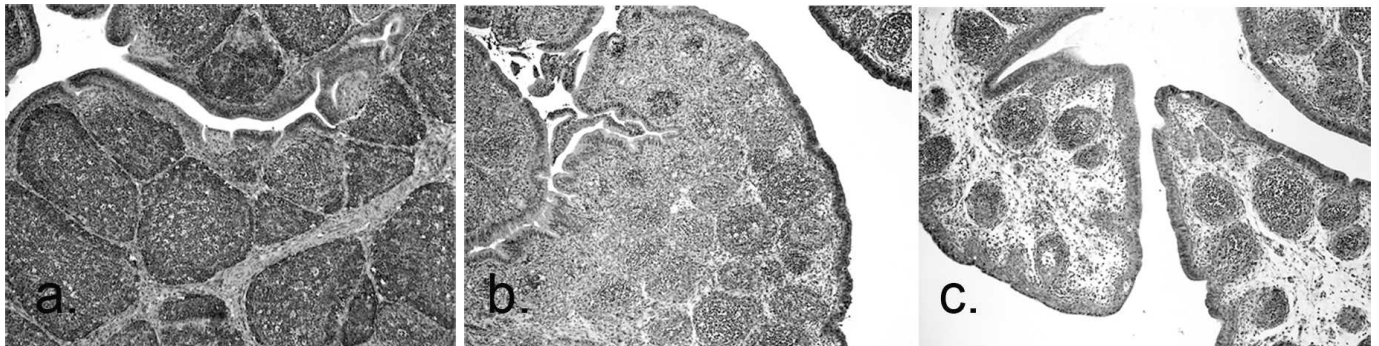


Fig. 2. (a) Normal bursa from a sham inoculated poult, 4 days PI. Hematoxylin and eosin. Magnification, 100 $\times$ . (b) Bursa with cortical and medullary follicle lymphoid depletion from poult inoculated with reovirus, 4 days PI. Hematoxylin and eosin. Magnification, 100 $\times$ . (c) Bursa with cortical and medullary follicle lymphoid depletion and severe stromal fibroplasia. Poult inoculated with rotavirus astrovirus and reovirus, 4 days PI. Hematoxylin and eosin. Magnification, 100 $\times$ .

Table 3. Results of TAsTV-2 RT-PCR testing of poultS inoculated with TAsTV-2.

Treatment Group	Days postinoculation				
	2	4	7	10	14
TAsTV-2	100 (10/10) <sup>A</sup>	100 (10/10)	100 (10/10)	100 (10/10)	40 (4/10)
TAsTV-2 + TRotV	90 (9/10)	100 (10/10)	100 (10/10)	100 (10/10)	60 (6/10)
TAsTV-2 + TReoV	100 (10/10)	80 (8/10)	100 (10/10)	100 (8/8)	87.5 (7/8)
TAsTV-2 + TRotV + TReoV	100 (10/10)	100 (10/10)	100 (10/10)	100 (10/10)	70 (7/10)

<sup>A</sup>Percent positive (number positive/total tested).

PI, the only poultS positive for TRotV were 20% (2 of 10) of the poultS in the group exposed to all three viruses. The group that was exposed to all three viruses was the only group in which there were at least some individual TRotV-positive poultS at all time points.

## DISCUSSION

The roles of different viruses in enteric disease have not been well defined, and in commercial flocks it is not uncommon to find clinically normal birds infected with enteric viruses. Frequently, one flock will be infected with numerous viruses (8,11,16,17). In order to sort out the effects of concomitant infection of young turkeys with two of the most common enteric viruses (TRotV and TAsTV-2) and a relatively common virus that is probably immunosuppressive (TReoV), 3-day-old broad-breasted white poultS were exposed to TRotV, TAsTV-2, and TReoV in all possible combinations.

Body weight was used as a primary measure of disease severity because of its importance in production and because it can be quantified. Differences were observed between the sham inoculates and the virus-exposed groups. Among the groups that were exposed to viruses, groups with TRotV and TAsTV-2 in any combination were more severely affected than TReoV alone. Furthermore, although differences among groups exposed to multiple viruses were not significantly different from each other, the group that was exposed to all three viruses consistently had the lowest body weights. This suggests that poultS exposed to all viruses in commercial conditions could perform more poorly than poultS exposed to fewer viruses, regardless of the severity of other clinical signs, which were similar among all TRotV- and TAsTV-2-exposed groups.

The lesions and clinical signs observed here (huddling, diarrhea, agitation) are consistent with numerous previous reports of enteric virus pathogenesis (1,2). However, in contrast to a synergistic interaction observed between rotavirus and enterovirus or astrovirus shown by earlier reports (5,15), the severity of clinical signs, microscopic lesions, and body-weight differences were not clearly enhanced when the birds were exposed to more than one virus. Also the effect of immunosuppression caused by TReoV appeared to be minimal when the birds were exposed to the viruses at the same time. Perhaps if the birds were exposed to TReoV prior to exposure to the enteric viruses there would be more of an impact because the effects of the bursal damage would be greater.

Cloacal shedding of TRotV and TAsTV-2 appeared to follow different patterns. TAsTV-2 was shed by a high proportion of poultS throughout the duration of the experiment, which is consistent with previous experiments with TAsTV-2-infected SPF poultS (10) and studies of commercial flocks in which TAsTV-2 seems to persist (10). In contrast TRotV was shed by 100% of the inoculated birds at 2 days PI, after which the proportion of infected poultS decreased through day 14 PI when only two of the birds exposed to all three viruses were positive. Corresponding with this, microscopic lesions observed in the intestines of TRotV-infected poultS at 4 days PI were mostly reparative in nature, with numerous immature enterocytes being produced in face of the loss of mature enterocytes, indicating that the damage occurred earlier. TRotV antigen staining was present in the enterocytes at the distal section of the affected villi and sloughed epithelial cells. In previous studies, we showed that TAsTV-2 and TReoV induced only mild microscopic lesions in the intestines, with mild damage to the intestinal epithelium and a mild inflammatory response (9,10). Viral antigen staining in tissues from poultS infected with TAsTV-2 was most commonly found in the enterocytes at middle section of the affected villi (10). Viral antigen staining in tissues from poultS infected with TReoV was present in the bursa in the surface epithelial cells and macrophages, and to lesser degree, in splenic red pulp macrophages and intestinal epithelial cells (9). This indicates a difference in the basic pathogenesis of these viruses which needs to be further evaluated.

These results suggest that interactions among these viruses may have some effect on body weights, although the interaction was not sufficient to reproduce the full clinical disease syndrome seen in the field. Additionally, the relatively high prevalence of TRotV and TAsTV-2 infection in turkey flocks regardless of flock disease status (8,16) indicates that other factors in the field, whether environmental, management, or microbial are necessary for the full clinical disease of PEC or PEMS to develop. For example, age at exposure may be a critical factor as Hayhow and Saif (5) reported more severe disease and synergism between an avian rotavirus and enterovirus when the poultS were exposed at 2 weeks *vs.* 3 days of age. Similarly, Yason *et al.* (25) also reported more severe lesions in older turkeys exposed to an avian rotavirus than in young poultS. Here we exposed the birds at 3 days posthatch in an attempt to simulate the early age at which poultS would be exposed to environmental virus when placed at the farm.

Alternatively, there may be natural pathotypic variants of the enteric viruses that produce more severe disease than the isolates used

Table 4. Results of TRotV RT-PCR testing of poultS inoculated with TRotV.

Treatment group	Days postinoculation				
	2	4	7	10	14
TRotV	100 (10/10) <sup>A</sup>	70 (7/10)	0 (0/10)	20 (2/10)	0 (0/10)
TRotV + TAsTV-2	100 (10/10)	50 (5/10)	10 (1/10)	40 (4/10)	0 (0/10)
TRotV + TReoV	100 (10/10)	60 (6/10)	25 (2/8)	50 (3/6)	0 (0/6)
TAsTV-2 + TRotV + TReoV	100 (10/10)	45 (4/9)	20 (2/10)	80 (8/10)	20 (2/10)

<sup>A</sup>Percent positive (number positive/total tested).

here. The particular astrovirus and rotavirus types that were utilized in this study were selected because they were relatively common (e.g., TAstV-2 is more common than TAstV-1) (11); only the TRoV TK/NC/SEP-44/03 isolate is known to be relatively virulent (4,20). Other considerations why disease in these experimental infections tends to be mild may be due to attenuation by cell-culture and egg propagation or to low challenge dose because accurate titration of some enteric viruses is difficult. Furthermore, in a laboratory setting, animal care facilities eliminate numerous environmental stressors, such as excessive cold, heat, dampness, or other pathogens that the birds may experience in the field that can nonspecifically contribute to disease severity through physiological stress. Because of these and other unknown factors, generalizations about the relative virulence of all TRoV and TAstV-2 strain can not be made. Further work needs to be done to elucidate the mechanism of TRoV and TAstV-2 pathogenesis and to understand their full impact on turkey production.

## REFERENCES

1. Barnes, H. J., and J. S. Guy. Poult enteritis-mortality syndrome. In: *Diseases of poultry*, 11th ed. Y. M. Saif, ed. Blackwell, Ames, IA. pp. 1171–1180. 2003.
2. Barnes, H. J., J. S. Guy, and J. P. Vaillancourt. Poult enteritis complex. Scientific and technical review. *World Organisation for Animal Health* 19:565–588. 2000.
3. Day, J. M., E. Spackman, and M. Pantin-Jackwood. A multiplex RT-PCR test for the differential identification of turkey astrovirus type 1, turkey astrovirus type 2, chicken astrovirus, avian nephritis virus, and avian rotavirus. *Avian Dis.* 51:681–684. 2007.
4. Day, J. M., E. Spackman, and M. J. Pantin-Jackwood. Turkey origin reovirus-induced immune dysfunction in specific pathogen free and commercial turkey poults. *Avian Dis.* 52:387–391. 2008.
5. Hayhow, C. S., and Y. M. Saif. Experimental infection of specific-pathogen-free turkey poults with single and combined enterovirus and group A rotavirus. *Avian Dis.* 37:546–557. 1993.
6. Heggen-Peay, C. L., M. A. Qureshi, F. W. Edens, B. Sherry, P. S. Wakenell, P. H. O'Connell, and K. A. Schat. Isolation of a reovirus from poult enteritis and mortality syndrome and its pathogenicity in turkey poults. *Avian Dis.* 46:32–47. 2002.
7. Lozano, L. F., A. A. Bickford, A. E. Castro, J. Swartzman-Andert, R. Chin, C. Meteyer, G. Cooper, B. Reynolds, and R. L. Manalac. Association of Reoviridae particles in an enteric syndrome of poults observed in turkey flocks during 1988. *J. Vet. Diagn. Invest.* 1:254–259. 1989.
8. Pantin-Jackwood, M., J. M. Day, M. W. Jackwood, and E. Spackman. Enteric viruses detected by molecular methods in commercial chicken and turkey flocks in the United States between 2005 and 2006. *Avian Dis.* 52:235–244. 2008.
9. Pantin-Jackwood, M. J., E. Spackman, and J. M. Day. Pathology and virus tissue distribution of Turkey origin reoviruses in experimentally infected turkey poults. *Vet. Pathol.* 44:185–195. 2007.
10. Pantin-Jackwood, M. J., E. Spackman, and J. M. Day. Pathogenesis of type 2 turkey astroviruses with variant capsid genes in 2-day-old specific pathogen free poults. *Avian Pathol.* 37:193–201. 2008.
11. Pantin-Jackwood, M. J., E. Spackman, J. M. Day, and D. Rives. Periodic monitoring of commercial turkeys for enteric viruses indicates continuous presence of astrovirus and rotavirus on the farms. *Avian Dis.* 51:674–680. 2007.
12. Reed, L. J., and H. Muench. A simple method for estimating fifty percent endpoints. *American Journal of Hygiene* 27:493–497. 1938.
13. Reynolds, D. L. Multi-causal enteric diseases. In: *Diseases of poultry*, 11th ed. Y. M. Saif, ed. Blackwell, Ames, IA. pp. 1169–1170. 2003.
14. Reynolds, D. L., and Y. M. Saif. Astrovirus: a cause of an enteric disease in turkey poults. *Avian Dis.* 30:728–35. 1986.
15. Reynolds, D. L., Y. M. Saif, and K. W. Theil. Enteric viral infections of turkey poults: incidence of infection. *Avian Dis.* 31:272–276. 1987.
16. Reynolds, D. L., Y. M. Saif, and K. W. Theil. A survey of enteric viruses of turkey poults. *Avian Dis.* 31:89–98. 1987.
17. Saif, L. J., Y. M. Saif, and K. W. Theil. Enteric viruses in diarrhetic turkey poults. *Avian Dis.* 29:798–811. 1985.
18. Simmons, D. G., W. M. Colwell, K. E. Muse, and C. E. Brewer. Isolation and characterization of an enteric reovirus causing high mortality in turkey poults. *Avian Dis.* 16:1094–1102. 1972.
19. Spackman, E., D. Kapczynski, and H. Sellers. Multiplex real-time reverse transcription-polymerase chain reaction for the detection of three viruses associated with poult enteritis complex: turkey astrovirus, turkey coronavirus, and turkey reovirus. *Avian Dis.* 49:86–91. 2005.
20. Spackman, E., M. Pantin-Jackwood, J. M. Day, and H. Sellers. The pathogenesis of turkey origin reoviruses in turkeys and chickens. *Avian Pathol.* 34:291–296. 2005.
21. Suresh, M., and J. M. Sharma. Pathogenesis of type II avian adenovirus infection in turkeys: in vivo immune cell tropism and tissue distribution of the virus. *J. Virol.* 70:30–36. 1996.
22. Theil, K. W., D. L. Reynolds, and Y. M. Saif. Isolation and serial propagation of turkey rotaviruses in a fetal rhesus monkey kidney (MA104) cell line. *Avian Dis.* 30:93–104. 1986.
23. Theil, K. W., and Y. M. Saif. Age-related infections with rotavirus, rotaviruslike virus, and atypical rotavirus in turkey flocks. *J. Clin. Microbiol.* 25:333–337. 1987.
24. Yason, C. V., and K. A. Schat. Pathogenesis of rotavirus infection in various age groups of chickens and turkeys: clinical signs and virology. *Am. J. Vet. Res.* 48:977–983. 1987.
25. Yason, C. V., B. A. Summers, and K. A. Schat. Pathogenesis of rotavirus infection in various age groups of chickens and turkeys: pathology. *Am. J. Vet. Res.* 48:927–938. 1987.
26. Yu, M., Y. Tang, M. Guo, Q. Zhang, and Y. M. Saif. Characterization of a small round virus associated with the poult enteritis and mortality syndrome. *Avian Dis.* 44:600–610. 2000.

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